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PERSPECTIVE

Emerging trends and prospects of electroconductive bioinks for cell-laden and functional 3D bioprinting

Harish K. Handral[1] · Vaishali P. Natu[2] · Tong Cao[2] · Jerry Y. H. Fuh[1,3] · Gopu Sriram[2,3] · Wen F. Lu[1,3]

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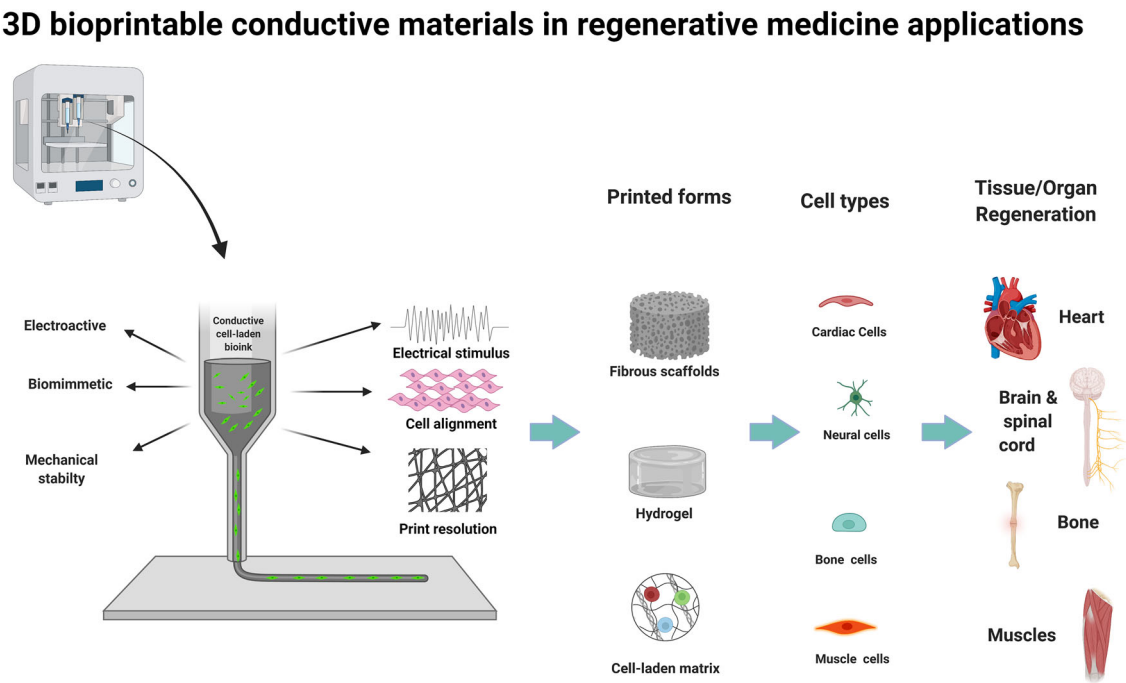
Graphic abstract



Introduction

3D bioprinting offers a unique biofabrication platform that allows the generation of functional tissue constructs in a spatially/geometrically controlled and automated manner using a 3D printer and bioink. Bioink serves as the carrier medium that provides the ideal physico-mechanical characteristics for printability, shape fidelity, and support; and a biological microenvironment for the living cells prior to, during, and post-printing [1]. These bioinks are typically expected to possess biocompatible and biofunctional characteristics that allow cellular viability, cell attachment, cell spreading, proliferation, cell–cell, and cell–matrix interactions [2]. In addition to the mechanical and biological characteristics,

✉ Gopu Sriram
dengs@nus.edu.sg

✉ Wen F. Lu
mpelwf@nus.edu.sg

1 Department of Mechanical Engineering, National University of Singapore, Singapore, Singapore

2 Faculty of Dentistry, National University of Singapore, Singapore, Singapore

3 NUS Centre for Additive Manufacturing (AM.NUS), Singapore, Singapore

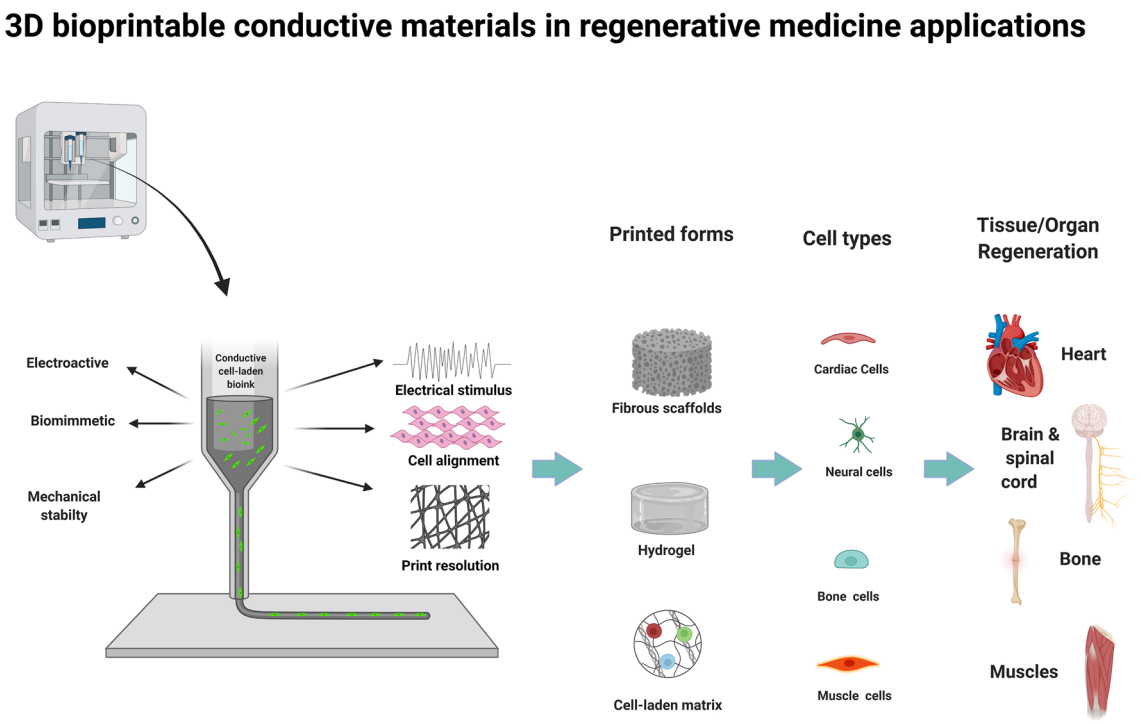


Fig. 1 Schematic illustration on the scope of 3D bioprintable conductive materials for cell-printing applications for organ/tissue regeneration

it would be ideal for the bioinks used for the fabrication of electrically active tissues such as neural, skeletal, and cardiac muscle tissues to possess electroconductive properties (Fig. 1). Electrical stimulation (ES) is a physical cue that plays a crucial role in cell signaling and functioning in these tissues. Along with biochemical and biophysical cues, ES also influences cell proliferation, differentiation for tissue repair, and regeneration. A lack of ES can lead to an adverse impact on a cell's functional features and eventually to cell death, specifically in cardiac, nervous, and skeletal muscle tissues [3]. Among biophysical cues—including surface topography, substrate stiffness, compression, and stretching—electrical and magnetic fields play a vital role in cellular metabolic functions and cell differentiation. Clinically directed studies using ES have demonstrated effective relief of pain, an enhancement of blood circulation, and a reduction in vascular and skeletal muscle tensions, thus highlighting the importance of ES for tissue repair and regeneration [4].

Conductive materials have been researched for tissue engineering applications for almost a decade, while the 3D printing of functionally active, conductive biomaterials is still in an early and exploratory phase. Polymers with intrinsic electroconductive properties have been widely used for flexible electronics, bioelectronics, and other applications requiring conductive properties. A basic feature of conductive polymers for propagating a charge is based on the movement of delocalized electrons through conjugated systems and the migration of electrons among neighboring redox sites via electron exchange channels [5]. Relying on unique polymeric features, such as electrical, mechanical stability, and even biocompatibility properties, strong interest in conductive polymers has appeared in the 3D-printing and healthcare industries [6]. Electroconductive biomaterials used for biomedical applications include polypyrrole (PPy), polyaniline (PANI), polyethylene dioxythiophene (PEDOT), carbon-based biomaterials, and metallic nanoparticles [7].

In this perspective review, we present recent developments in biomaterials with conductive properties for 3D-printing applications, strategies for formulating viable conductive bioink for the fabrication of biomimetic 3D-bioprinted cell-laden conductive tissue constructs, and future outlooks for tissue engineering and regenerative applications.

Conductive biomaterials for 3D bioprinting

Conductive biomaterials can be used in the form of conductive polymers or conductive fillers to obtain a favorable environment for supporting cellular activities. Conductive fillers impart conductivity to non-conductive materials such as gelatin, chitosan, polycaprolactone (PCL), and others [8]. Carbon-based fillers such as carbon black, carbon nanotubes, carbon nanowires, and graphene, and metal-based fillers such as gold, platinum, and silver nanoparticles have been deeply explored due to their properties including tensile strength and

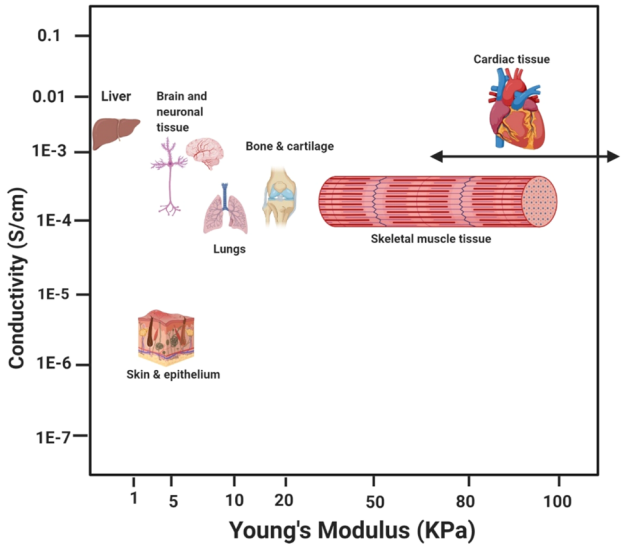


Fig. 2 The properties of the conductive platform can be modified for different tissues. The graphical plot provides guidance on the selection of biomaterials for the target tissue, taking into account the respective conductivity and mechanical properties. Adapted and recreated with permission from [8] (Created using Biorender.com)

electrical conductivity [9]. Their application is restricted due to pitfalls such as non-biodegradability, long-term in vivo toxicity, and the uneven distribution of these conducting particles. "Conducting polymers" is an umbrella term used for a group of materials that provide a biocompatible environment along with ES that promotes varied cellular activities such as proliferation, migration, differentiation, and adhesion and have proven to be efficacious in neural and cardiac tissue engineering [10]. Materials with intrinsic electrical conductivity have gained exponentially increasing interest in biomedical applications, such as in biosensors, drug delivery systems, biomedical implants, and tissue engineering. Conductive materials offer great adaptable mechanical properties for the target organs/tissues based on their usage in encapsulating specific cell types and biological materials and can be fabricated to enhance regenerative properties (Fig. 2). Commonly used conductive polymers with biological activity are as follows (also shown in Table 1).

Polypyrrole (PPy)

PPy is one of the most commonly used conductive polymers for biological applications owing to features such as higher conductivity (p-type conduction), biocompatibility, ease of synthesis, and stability [11]. PPy polymer-based, electrospun fibers have been evaluated for biocompatibility as well as conductivity in neuronal tissues both with in vitro and in vivo platforms [12]. Some of the limitations of PPy, such as rigidity, insolubility, and poor degradation features, hinder post-printing steps, especially for biological applications [13]. The poor solubility of PPy renders it not suitable for use in traditional fabrication methods such as electrospinning, and its non-degradable feature limits its use in

Table 1 Different conductive materials used in cell-laden bioprinting and their applications in tissue engineering

Conductive material	Main component of hydrogel	Gelation/crosslinking method	Cell type	Application
Gold nanoparticles	GelMA and Alginate	UV and calcium chloride respectively	Cardiac fibroblasts	Cardiac tissue engineering [51]
Gold nanowires	Type I collagen	Genipin solution	C2C12 myoblast cells	Muscle tissue regeneration [60]
PPy nanoparticles	Type I collagen	PEG buffer solution	Pheochromocytoma (PC12) cells	Neural regeneration [61]
Block copolymer of PPy and PCL	Type I collagen	Incubator at 37 °C	Pheochromocytoma (PC12) cells	Neural differentiation [62]
Poly(3,4-ethylenedioxythiophene): poly(styrenesulfonate) (PEDOT:PSS)	GelMA	Calcium chloride and visible light	C2C12 myoblast cells	Bioink development [39]
	Methylcellulose and kappa-carrageenan	Potassium chloride solution	Human embryonic kidney 293 (HEK-293) cells	Bioink development [63]
Carbon nanotubes (CNT)	GelMA	UV	NIH-3T3 fibroblasts and human mesenchymal stem cells	3D ECM scaffolds [64]
	Cellulose nanofibrils	Air drying	SH-SHY5Y human neuroblastoma cells	Neural tissue engineering [65]
Graphene/graphene oxide	Polyurethane gel	Temperature at 37 °C	Neural stem cells	Neural tissue engineering [57]
	Alginate	Calcium chloride	Mesenchymal stem cell	Bone tissue engineering [66]

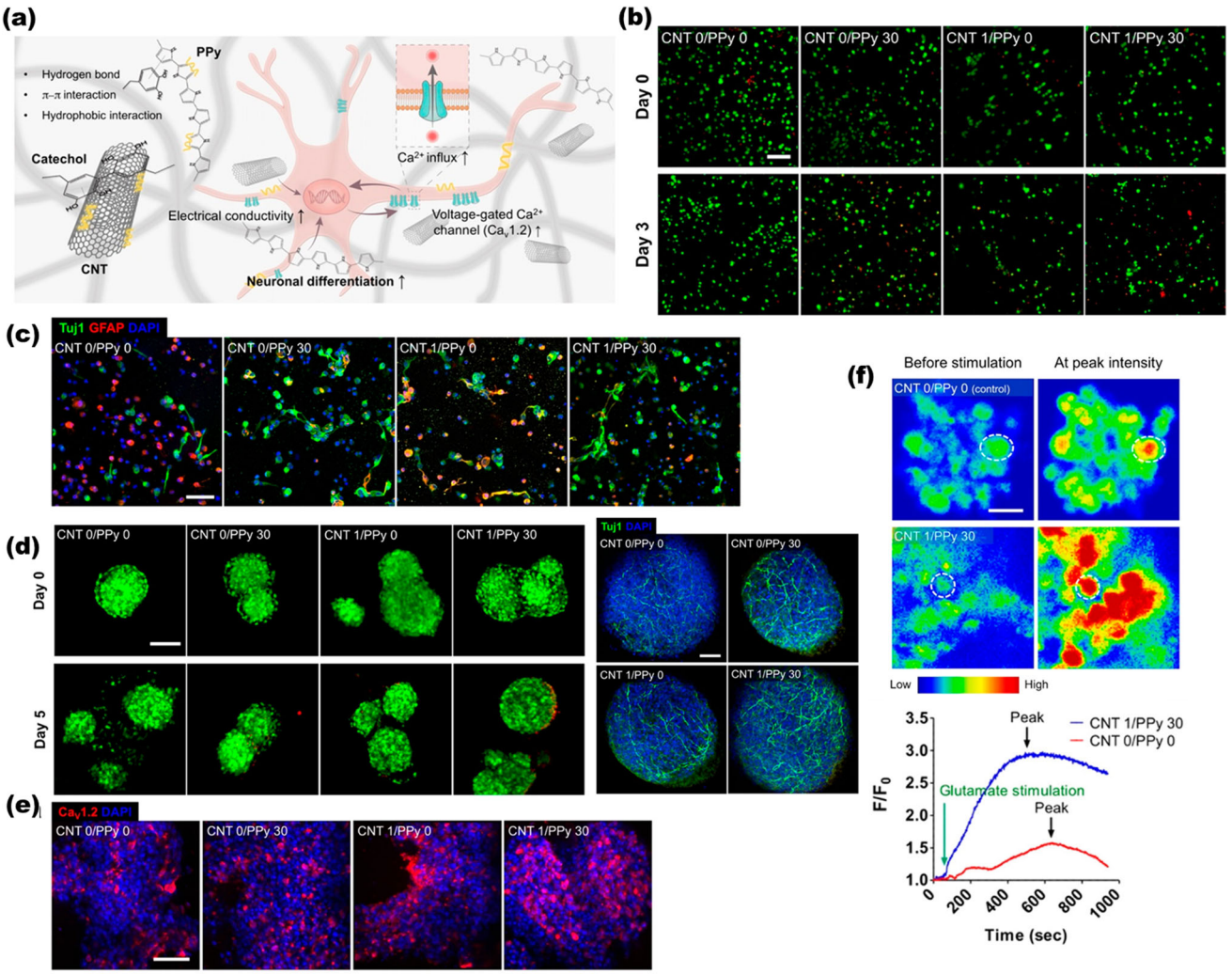


Fig. 3 Overview of cell-laden conductive hydrogel studies focusing on neuronal differentiation. **a** Schematic showing the impact of conductive material on neuronal differentiation. **b** Live/Dead analysis neural stem cells (NSCs) in conductive hydrogels and **c** expression of neuronal and astrocyte marker by NSCs in conductive hydrogel. **d** Live/Dead analysis of NSC neurospheres encapsulated in conductive hydrogels and neurospheres expressing a neuronal marker. **e** Immunostaining of L-type voltage-gated calcium channel ion (Cav1.2) expression and glutamate-responsive calcium influx in hiPSC-NPCs encapsulated in electroconductive hydrogels. **f** Glutamate stimulation highlighting the intracellular Ca^{2+} influx in hiPSC-NPCs encapsulated in non-conductive hydrogel (CNT 0/PPy 0) and in electroconductive hydrogels (CNT 1/PPy 30). Scale bar in (**b**–**e**) = 50 μm; in (**f**) = 20 μm Adapted from [21], Copyright 2017, with permission from American Chemical Society

translational applications. Such drawbacks have been overcome by developing a conjugation of natural and synthetic composites [8]. Efforts have also been made to enhance the biofunctional properties of conductive polymers by blending them with natural and synthetic hydrogels and/or polymers. For instance, PPy has been incorporated with alginate, collagen, gelatin, and other, known, biofunctional materials with the aim of acquiring biofunctional conductive polymer composites [14]. PPy nanoparticles combined with polylactic acid (PLA) have been shown to form PPy/PLA composite nanofiber-based films with good biocompatibility for cell growth and differentiation, especially in Schwann cells along with human mesenchymal stem cells promoting sciatic nerve repair in rat models [12]. A biomaterial composite conjugated with electroactive polymers such as PPy allows the fabrication of biomaterial scaffolds with accurate geometry and size. Attempts have been made to use PPy-gelatin composites for neural tissue regeneration by investigating cell adhesion, distribution, and viability [15]. PPy-based hybrid biomaterials demonstrated superior cell–cell communication, and cellular metabolic activities which promoted neurogenesis, with and without ES [15–17] (Fig. 3). Culture of neuronal cells seeded over PPy-based hybrid scaffolds in the presence of ES show longer neurite extension compared with non-stimulated controls. Besides having these potential advantages, its intrinsic brittle nature has limited its applications for soft tissue engi-

neering and cell-laden tissue printing. PPy scaffolds are known to regulate drug release with changes in pH. This phenomenon has been applied in bone repair and regeneration since fluctuations in pH in bone regenerating areas are common, occur naturally, and can trigger PPy for drug delivery applications. PPy nanoparticles blended with polyethylene (PEG) were 3D printed and tested for biocompatibility for their potential use in patient-customized bone scaffolds that can aid natural bone repair and growth [18, 19]. PPy-based injectable scaffold conjugation with other polymers has been demonstrated to address cardiac tissue repair by mimicking elastic and conductive properties to provide a feasible method to repair cardiac tissue [20].

Polyaniline (PANI)

PANI is the second-most widely used conductive polymer and is commonly referred to as black aniline. PANI is one of the well-characterized conductive polymers and has a range of structural types, improved stability, and ease of charge-transport capabilities that are critical for tissue engineering applications to stimulate electrical conduction [22]. It is classified into three forms depending on the degree of oxidation. These are given as follows: the pernigraniline base is fully oxidized, the base of emeraldine is semi-oxidized, and the fully reduced form is known as the base of leucoemeraldine. The most stable and conductive is PANI emeraldine [23]. PANI is able to regulate high-stress conditions by eliminating free radicals, has antibacterial effects, and is possibly the only conductive polymer that has an adjustable electrical characteristic [24]. PANI has various advantages compared to other conducting polymers, such as a low cost, it is easily synthesized, and it is able to shift between resistive and conducting conditions electrically [25]. PANI has also demonstrated its support for cell growth and differentiation, and has also been used for scaffold fabrication studies. The main limiting factor, however, is the non-biodegradability of PANI-based scaffolds, which can cause inflammation and contribute to additional surgery in order to avoid degradation [26].

Biocompatibility, cell adhesion, cell differentiation, growth, and cellular morphologies were studied in neural stem cells cultured on PANI-PCL scaffolds [27]. PANI used with chitosan has also highlighted promising effects on neural differentiation [28]. A chitosan-polyaniline hydrogel was used with PC12 cells. Chitosan-PANI hydrogel provided a hierarchical topographical surface to induce neural differentiation. The impact of spatial orientation of the conductive material was confirmed on neural differentiation by comparing with non-patterned substrates [29]. Significant changes were showed by PC12 cells grown on patterned conductive substrates when compared to standard 2D-tissue culture flasks, specifically in gene expression and in neural differentiation abilities [30]. Along with biomimetic properties, neural differentiation features have been exhibited by conductive chitosan-polyaniline scaffolds. Hierarchical and structured tissue architecture is important for retaining the functional features of bioengineered in vitro tissues, especially tissues such as skeletal muscle and cardiac muscle tissues, in which cellular alignment, cell–cell junctions, and conductivity play a major role in cell/tissue functioning. Conductive polymers combined with hydrogel-based biomaterials have demonstrated regeneration of peripheral nerve repair. The use of polyacrylamide-based biocompatible polymers along with conductive polyaniline (PANI) to develop a mechanically stable, biologically active hydrogels with conductive functionality has supported the restoring and repair of sensory functions of neuron cells [31]. PANI combined with PCL has yielded interesting results in enhancing functional properties, more specifically, providing the appropriate guidance cues for cells to modulate cell behavior and cell alignment. The alignment of PANI-PCL nanofibers has promoted cellular behavior that could guide myoblast orientation and promote myotube formation highlighting the synergistic effects of topographical and electrical cues [32]. Human adipose-derived stem cells cultured on 3D-printed conductive polymeric composite scaffolds of PANI and PCL have been assessed for in vitro cytocompatibility, cell viability, and proliferation up to 21 days for bone tissue engineering. The morphological, mechanical, conductivity, and preliminary biological properties of these conductive scaffolds for bone tissue engineering applications have been additionally demonstrated [33]. PANI has also been demonstrated to induce cellular alignment and elongation of skeletal muscle cells to aid the formation of muscle-like structures [34].

Poly(3,4-ethylenedioxythiophene) (PEDOT)

PEDOT is a biocompatible conductive polymer that has been used for biomedical applications. PEDOT shares characteristics similar to those of polythiophene derivatives and melanin, which are naturally bioactive materials [35]. PEDOT has greater advantages for bioink formulation, since its monomer is of a hydrophilic nature that makes it easily soluble in water and can be blended with other materials in synthetic aqueous systems [36]. The doping of PEDOT into poly styrene sulfonate (PSS) can form thin film-like surfaces/coatings. PEDOT:PSS conductive composites blended with arginine-glycine-aspartate (RGD) peptides promoted cell proliferation and hemocompatibility (human serum absorption), showing the biocompatibility and enhancement of tissue regenerative features [37]. Thus, PEDOT:PSS 3D-printed surfaces with bioactive peptides could have potential applications in cardiovascular implants. Attempts have been made to formulate conductive bioinks with tunable thixotropic properties by blending methylcellulose and kappa-carrageenan (MC/kCA) hydrogels with PEDOT:PSS

conducting polymers [38]. Varying the ratios of MC and kCA resulted in the potential to tune the thixotropic behavior of the formulated bioink and obtain high shape fidelity without a secondary support bath [39]. The cytocompatibility of the bioink was evaluated using Human Embryonic Kidney-293 (HEK-293) cells by live-dead assay. In addition to the bioactive profile, the mechanical stability of the printed constructs has also been demonstrated, which is one of the key features of fabricating complex 3D structures of tissue/organ for tissue engineering applications.

Carbon-based conductive bioink

Carbon-based nanomaterials have been often integrated into cell-laden bioinks due to their excellent electric and mechanical characteristics, in particular for applications in neural and muscle tissue engineering [40]. Graphene oxide (GO), a representative of oxidized graphene, is a blend of hybridized carbon atoms sp2 and sp3 with a thin graphite layer covalently bound to functional groups containing oxygen [41]. A substantial increase in oxygen metabolism and neural differentiation was observed in polyurethane (PU) hydrogels containing GO components [42]. In another study, the patterned architecture of 3D, bioprinted gelatin methacryloyl (GelMA)/graphene bioinks demonstrated enhanced neural regeneration and proposed the use of hybrid graphene constructs for multi-responsive 4D bioprinting aimed at the fabrication of smart, nerve, conduit-like patterns [43]. In addition, GO blended with a GelMA/PEGDA matrix has shown promising results in chondrogenic differentiation and bone regeneration applications [44]. The fabrication of graphene-PCL scaffolds with a controlled size, shape, and pore distribution by 3D printing demonstrated substantial cell adhesion when human adipose-derived stem cells were bioprinted [45]. Similarly, PCL-reduced graphene oxide (PCL-RGO) bioprinted with PC12 cells has shown promising cell growth and differentiation in the case of peripheral nerve injury [46]. Carbon nanotubes are either used as substrates or as additives for cardiac tissue regeneration due to their excellent mechanical and electrical properties [47] (Fig. 4).

Nanoengineered conductive bioink

The integration of inorganic nanoparticles with sufficient biocompatibility and electro-conductivity properties into a bioink can provide a range of cell-printing advantages, including: (a) an increased electroconductive surface area, (b) improved cytocompatibility, (c) enhanced biomimetic topography, (d) improved mechanical stability, and (e) ease of printability [49].

Gold nanoparticles (AuNPs) represent an emerging class of nanomaterials for tissue engineering because of their various advantages in terms of biocompatibility, electrical conductivity, and chemical stability [50]. AuNPs have high electrical conductivity, a range of geometries (i.e., nanospheres, nanotubes, and nanowires), inherent optical properties, ease of surface functionalization, and satisfactory cytocompatibility. For this purpose, AuNPs were combined with different bioactive materials in order to formulate electroactive bioinks. Gold nanorods (GNRs), mixed with GelMA for formulating nanocomposite bioink were used and were shown to be both cytocompatible and feasibly used for printing functional cardiac tissue constructs [51]. Additionally, GelMA-GNRs enhanced the electric stimulation of cardiac cells and improved cellular functionality in printed cardiac patches.

Carbon-based nanomaterials, such as carbon nanotubes (CNTs), graphene, and its chemical derivatives, have emerged as a promising new class of NMs for stem cell-based tissue engineering and regenerative medicine. These carbon-based nanomaterials are electrically conductive, biocompatible, have a large surface area with good mechanical properties, and have rapid mass and electron transport kinetics, all of which are essential for the chemical/physical stimulation of differentiated cells [52, 53]. CNTs embedded in scaffolds have been shown to improve cardiac cell adhesion, viability, and maturation while also offering functionality for cardiac tissue applications [47]. Nanostructured composites of hydroxyapatite-based scaffolds provide a closer structural support approximation to native bone architecture for cells and regulate cell proliferation, differentiation, and migration, which results in the formation of functional tissues [54]. Nanoengineered bioink formulation for bone formation with 3D bioprinting has allowed for precision-based printability, mechanical features, and a rate of degradation that enables the customized fabrication of mechanically strong and cellularized structures [55].

Bioprintable, cell-laden electroconductive bioinks

Despite a sizeable amount of work on 3D printed conductive scaffolds, there is minimal work available in the literature on cell-laden electroconductive bioink. This is owing to the challenge of incorporating cells within hydrogel bioinks with electroconductive components. The challenge with 3D bioprinting for developing functionally active tissue constructs arises from the low, electrical, conductive properties of most of the bioinks currently available and from the polymers used in bioinks that lack inherent conductive properties. Electroconductive hydrogels have shown the delivery of consistent electrical cues to cells encapsulated in 3D constructs, thereby promoting cell growth and differentiation [56]. Conductive nanomaterials, such as metallic or carbon-based nanomaterials, have been found to be useful in the development of conductive bioinks. Some of the interesting studies on the

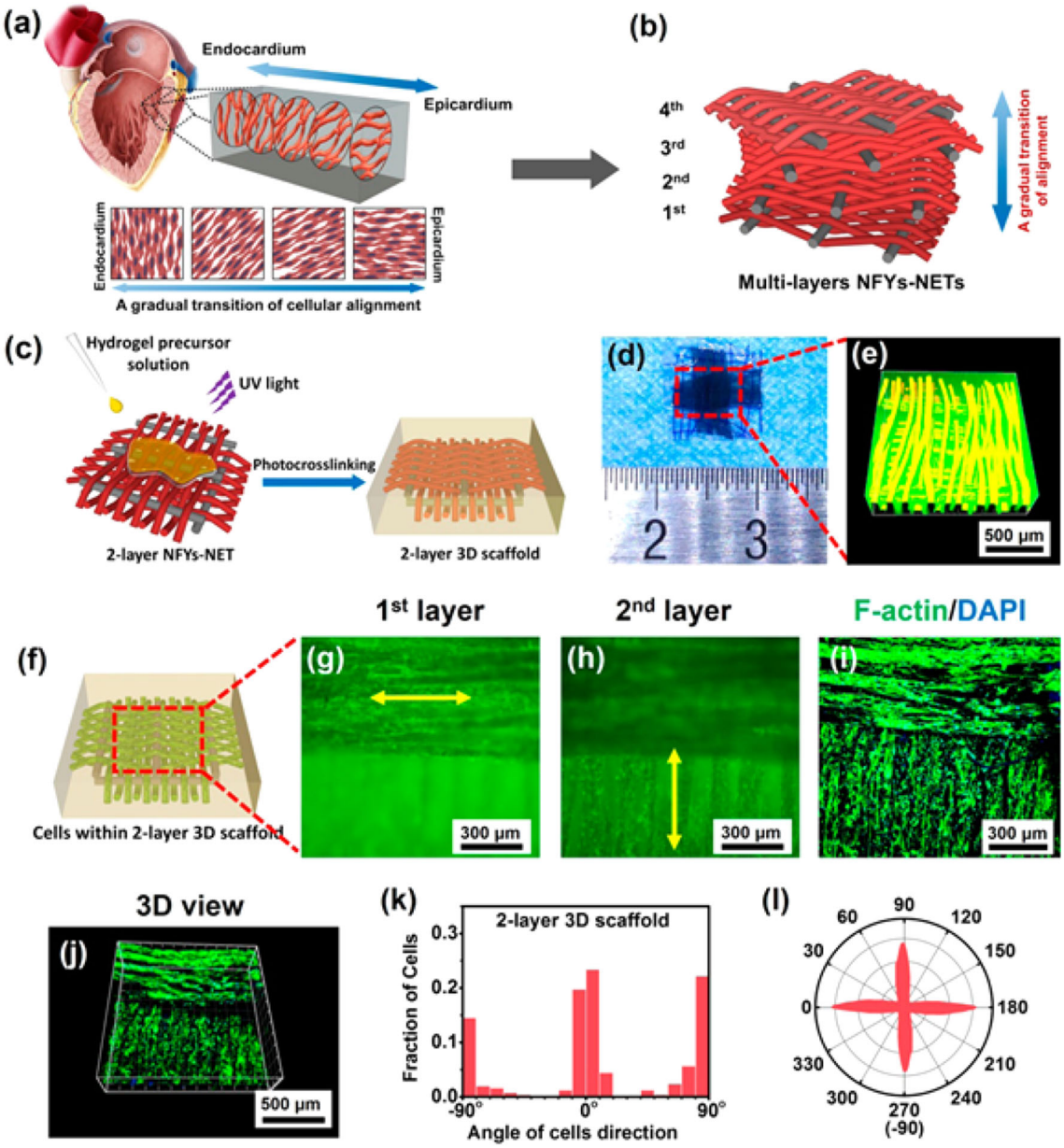


Fig. 4 Generation of 2-layered, 3D, Nanofiber Yarns Network (NFYs-NET)/Gel scaffolds and embedded cardiomyocytes in these 3D hybrid scaffolds. **a** Illustration of myocardium cells showing a transition in alignment from endocardium to epicardium. **b** Schematic illustration of aligned layers and orientation of NFYs-NETs. **c** Fabrication of 2-layered NFYs-NET with an orthogonal orientation within GelMA hydrogel. The gross image **d** and 3D view of the confocal image **e** of the 2-layer 3D scaffolds. **f** Cells cultured on two NFYs-NET layers within a hydrogel shell. Cardiomyocytes stained with F-actin (green) in the NFYs-NET layer with a horizontal direction **g**, vertical direction **h**, top view **i**, and 3D view **j**. **k**, **l** Cellular orientation and distribution was quantified to show that the cells aligned on one layer are perpendicular to the cells on the other layer. Reprinted from [48], Copyright 2017, with permission from American Chemical Society

bioprinting of cell-laden conductive hydrogels are listed in Table 1, and few of them are discussed below.

Graphene, a single layer of carbon atoms, has recently received much attention because of its distinct electrical and mechanical properties. The 3D printing of neural stem cells (NSCs) incorporated within a nanocomposite bioink consisting of thermo-sensitive aqueous polyurethane and graphene has been reported [57, 58]. Scientists have found that adding small amounts (25 ppm) of graphene to bioink has enhanced neuronal differentiation. Similarly, gold nanorods combined with GelMA and alginate form a printable conductive bioink that allows the 3D printing of structures using coaxial extrusion with calcium chloride as a crosslinking agent [51]. The addition of gold nanorods has shown enhanced cell adhesion and electrical signal propagation among cardiac cells by imparting electrical signals within the GelMA/alginate

bioinks. In another study, Rastin et al. [59] used a blend of methylcellulose and kappa-carrageenan (MC/kCA) hydrogels incorporated with PEDOT:PSS as a conductive biomaterial to formulate cell-laden bioinks. The authors used different blends of MC/kCA to modulate the thixotropic properties, bioprintability, and shape fidelity, and different weight ratios of PEDOT:PSS to modulate the electroconductive properties of bioink. Though in-depth functionality studies have not been carried out, the authors demonstrated excellent cell viability within the different blends of cell-laden electroconductive bioinks.

Topographical and electrical cues could be utilized to direct the orientation of electroconductive biomaterials within a cell-laden bioink, which in turn could enable the alignment of the cells within the bioprinted construct. Kim et al. [60] utilized the geometric shape and shear stress induced by a micro-size nozzle and the application of electrical fields post-printing to direct the orientation and alignment of gold nanowires (Au-nanowires) incorporated within a collagen-based, cell-laden bioink. The orientation of Au-nanowires provided contact guidance and an asymmetric electrical microenvironment leading to a parallel alignment of myoblasts with oriented actin fibers and multinucleated myotubes. This strategy was also shown to translate the upregulation of the muscle tissue regeneration upon transplantation. Similar studies using PEDOT nanoparticles incorporated within a cell-laden GelMA-based bioink demonstrated the enhanced myogenic differentiation potential of C2C12 cells in cell-laden, 3D-bioprinted muscle tissue constructs [61]. The GelMA-PEDOT bioink formulation showed excellent bioprintability and electroconductive properties, and in the presence of ES, the constructs showed greater proliferation and differentiation of C2C12 cells than those encapsulated in GelMA. Overall, these studies and those presented in Table 1 demonstrate the potential of cell-laden electroconductive bioinks for cell encapsulation and biomimetic tissue reconstruction.

Key factors to consider in the formulation of a conductive bioink

Standardization of the flow rheology

Conductive biomaterials for 3D bioprinting must match the flow behavior, sol–gel transition, as well as the viscoelastic response to meet the rheological and gelation kinetics of a bioink [67]. The regulation of these parameters is also crucial to avoid printing-induced damage to the cells to enable high cellular viability. The ability to refine these parameters is of strong importance to achieve a robust conductive bioink, something that is possible by the screening and characterization of conductive biomaterials. The use of additives or varying monomer concentrations is a commonly used approach to refining the viscosity of bioink [68]. An ideal conductive bioink must match the appropriate flow both during the printing and right after the printing. Shear-thinning bioinks, such as GelMA and others, are designed for addressing printability, shape retention, as well as cell viability during bioprinting [69]. Different bioink gelation mechanisms, including physical and chemical crosslinking strategies are applied to achieve the printable properties in hydrogels/biomaterials having different types of non-Newtonian behaviors [70]. Similarly, it is crucial to consider post-printing rheological properties such as shape fidelity and rapid gelation kinetics.

Tunable matrix mechanics

The recent development of 3D bioprinting has also highlighted biophysical features such as the mechanics and topography of the matrix and/or the biomaterial's impact on cell survival, functionality, as well as differentiation capacities. Human organs are made of multiple cell types and are composed of an extracellular matrix (ECM), displaying spatial variations and biomechanical properties and an elastic modulus with diversity ranging from 100 to 100,000 Pa [71]. Therefore, biomaterials which can be regulated and possess tunable mechanical properties to mimic physiological, in vivo-like ECM features are of high value for fabricating cell-laden 3D-printed tissues/organs. Biomechanical factors can impact cellular behaviors, such as proliferation, migration, and differentiation [72]. Adaptable biomaterials offer potential solutions for cell dispersion in hydrogels/biopolymers, allowing intercellular connections that promote cell migration, differentiation, and ultimately leading to the bio-functional activity of 3D-printed tissue constructs [73]. The development of smart bioinks with tunable characteristics including conductive properties would enable efficient applications in cell-laden bioprinted tissues, especially for neural, skeletal muscle, and cardiac tissue engineering applications [74].

Engineering the biochemistry

The choice of bioink components is primarily based on biocompatibility, molecular recognition, and functionality. Incorporating biomimetic chemicals—such as proteins, peptides, and growth factors—would enhance the essential cell supportive microenvironment for the regulation of cell growth and differentiation [75]. Being biocompatible is a minimum requirement for usefulness in bioprinting applications. The bio-functionalization of 3D-printable materials can be achieved using motifs, nanomaterials, and conductive materials that can aid ES and conductivity [2]. An interesting concept of using DNA-based peptides in bioink either as the

polymer backbone or even as a crosslinker would provide next generation bioinks which can be tunable for the rheological, biochemical, and even mechanical features of the 3D printed constructs [76]. Prospectively, biomimetic DNA peptides can be used for developing conductive biomaterials and bioinks. Recently, a study showed the formation of filamentous conductive nanowires from metal-reducing bacteria such as *Geobacter sulfureducens* [77]. Depending on molecular recognition and application, the tunable property of material functionality plays an important role in matching the requirement of the cells to mimic the artificial tissues.

Bio-functional puzzle and reinforcement strategies

A key challenge of bioprinting is identifying a functional material for developing printable bioink. This is critical for 3D bioprinting, especially when cells are laden inside the bioink and eventually grow and differentiate in the printed structures. Natural, hydrogel-based materials are an effective choice to consider for bioinks because they possess native characteristics that provide a hydrated and permeable 3D microenvironment for cell-adherence and metabolic activities. Moreover, natural, hydrogel-based biomaterials are in use for tissue engineering, disease modeling, drug delivery, and biomedical applications [78]. However, the requirements and aspects of 3D bioprinting, such as pH, temperature, pressure, physical forces, stress, strain, and rheological factors, limit the use of available hydrogels as bioprintable materials. A functional conductive bioink must satisfy the stringent requirement of bioprinting concepts. Importantly, a widely accepted bioink must be able to mimic the biophysical and biochemical features of the ECM, as illustrated in Fig. 5. Along with conductive features, printability, cytocompatibility, and biodegradability need to be considered for a potential conductive bioink [56].

The conductive feature of bioinks will be critical factors for excitable tissues such as cardiac, skeletal, muscle, neural, as well as smooth muscle tissues. The incorporation of conductive materials in printable bioink would enhance the functional insulating bridge among the pores of the scaffold, thus coupling the disconnecting walls among the cellular modalities in the printed tissue [74]. The application of ES to the printed tissues can activate the cellular population via the conductive factor of the material. Conductive materials, such as gold, carbon nanotubes, or graphene, have been used in 3D-printed scaffolds to stimulate conductivity among the cells in the scaffolds [79].

Extrusion-based bioprinting modalities

The principal and mechanism of extrusion bioprinting are similar to the rapid prototyping of polymer-based, fused deposition modeling (FDM). Similar to FDM, the hydrogel-based bioink is extruded using computer-assisted devices for modeling shape and geometry, upon which the shape of extruded hydrogel precursor is fixed using a crosslinking step which can be based on pH, temperature, ionic crosslinking, photochemical reactions, enzymatic, or guest–host induced chemical interactions [80].

This technology uses a series of automated engines, a print bed, a movable printer nozzle, and a system to deposit bioink (biomaterial with cells). This system can be digitally monitored on a computer at the exact time and place. A brief schematic illustration in Fig. 6 explains that extrusion printing can also be guided by many methods including, pneumatic pressure-based control, mechanical control, or piston-based control. Acellular or cell-loaded bioinks can therefore be imprinted on the print bed in a layer-by-layer pattern. The printing of cell-laden bioinks generally relies on pneumatic and piston-driven systems, while screw-driven systems are used for printing highly viscous materials such as thermoplastic polymers, e.g., PCL. Cell-laden hydrogel can be casted on the pre-printed thermoplastic molds to obtain mechanically stable structures. For printing lower-viscosity materials, pneumatic and piston-driven systems are used. Most commercial bioprinters are based on a pneumatic system with versatile options however, with restricted control of the deposition of inhomogeneous bioink, especially in the case of multi-material composites. To regulate the deposition of a variety of inks and biomaterials, such systems are most often flexible and can be configured with several printheads, cooling and heating systems, and light sources to regulate crosslinking as well as gelation [81]. However, the switching of multiple cartridges is important for mixing different inks efficiently within a single print design and for meeting the accuracy of interfaces and gradients of various materials and cell types.

Even when bioprinters have printheads with accuracies of 5 to 100 μm in the *x*–*y*-*z* planes, the tissue resolution of the printing structure is mainly based on an ink's rheology and the diameter of the nozzle. The resolution for cell-laden printing is generally limited to a maximum of 100 μm to millimeters, as shear stress at the nozzle tip has direct repercussions on a cell's viability and corresponds inversely with nozzle diameter [82]. In contrast, the printing of acellular biomaterial inks gives much higher resolutions, since cell viability limitations do not exist, and can be extruded at high pressures and small nozzles. Extrusion bioprinting is versatile for bioink compatibility and different cell culture models, such as cell-spheroid suspension, decellularized ECM solutions, hydrogels, and a wide range of viscosity biomaterials which could provide stronger mechanical support to the printed tissue construct. Owing to the above advantages, extrusion-based bioprinting is commonly used for tissue engineering and regenerative medicine applications.

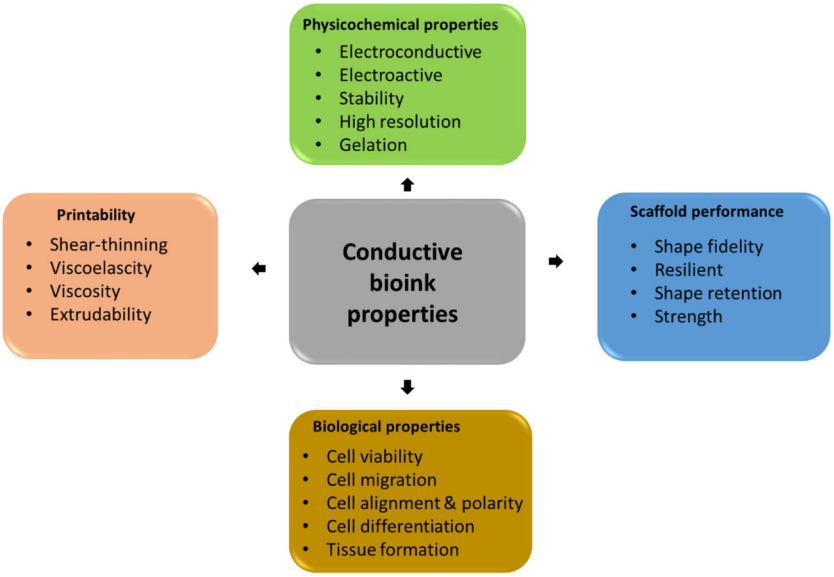


Fig. 5 Properties of a conductive bioink for bioprinting cell-laden conductive tissue constructs

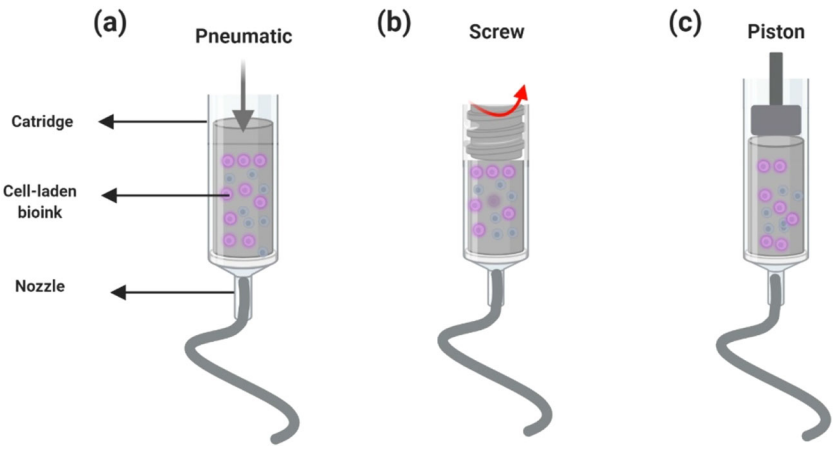


Fig. 6 Different types of extrusion bioprinting highlighting: **a** an air-pressure driven pneumatic system; **b** a mechanical rotation screw-driven printing system; **c** a mechanical force-driven piston printing system (Created with BioRender.com)

Applications of conductivity on bioengineered tissues/organs

The effect of conductive hydrogels on cell behavior (without ES)

Electrical cues can be delivered to cells locally by the use of electrically active materials in the form of scaffolds or hydrogels that encapsulate the cells. Conductive hydrogels are also called smart hydrogels as they are a combination of electrical conductivity and ECM-like properties of the hydrogel that are optimal for tissue regeneration [83]. Electroactive scaffolds of silk fibroin and conductive poly(aniline-co–N-(4-sulfophenyl) aniline) (PASA) at different concentrations enhanced the myogenic differentiation of C2C12 myoblast cells. The number and length of the myotubes were higher when the concentration of PASA was increased. In addition, the higher PASA content also contributed to the enhanced expression of myogenesis-related genes [84]. Cardiomyocytes cultured on conductive scaffolds made of aligned nanofiber yarns containing PCL, silk fibroin, and carbon nanotubes showed alignment, elongation, and the interconnection of the cardiomyocytes between the layers of the scaffold, and these cardiomyocytes expressed a mature phenotype with synchronous beating. When these scaffolds were encapsulated within a GelMA hydrogel shell laden with endothelial cells, it mimicked an endothelized myocardium containing aligned and elongated cardiomyocytes [48]. Conductive hydrogels made from alginate and varying concentrations of PPy were used to culture human bone marrow-derived mesenchymal stem cells. The cells exhibited a neural phonotype appearing elongated and larger after 14 days in culture and the proliferation of the cells was directly proportional to the concentration of PPy added. The differentiated cells also expressed early and late neurogenesis markers such as Tuj1 and MAP2 [85].

Implications of electrical conductivity on cell alignment and migration

The regenerative and self-renewal capacity of cells plays vital roles for tissue engineering and the repair of damaged tissues/organs. The regulation of cellular function from a clinical perspective is important for replicating in vivo-like physiological conditions, including cell migration, proliferation, differentiation, and other vital cellular processes. In terms of 3D-cell culture and bioprinting aspects, the choice of scaffold materials, biomaterial, as well as surface topography and other stimulating factors could play a role in tweaking cellular behavior [86]. Various studies have demonstrated the role of biochemical and biophysical factors influencing cellular behaviors. Among biophysical cues, ES has demonstrated a reduction in vascular tension, enhancing blood circulation, and even promoting the reabsorption of edema as well as joint fluid in clinical scenarios [87]. Besides these applications, ES has enhanced the neurite outgrowth from neural stem cells (NSCs) and was shown to promote differentiation. ES triggers intercellular signaling via intrinsic pathways, leading to an impact on cell migration, differentiation, and proliferation capabilities [88]. Thus, ES could provide a promising platform to alleviate some challenges of tissue engineering by enhancing the functional aspects of bioengineered tissues.

As discussed earlier, various materials and polymers are widely used for inducing conductivity in cell culture platforms. As depicted in Fig. 7, these methods are most commonly used to induce conductivity through, direct coupling, capacitive coupling, and inductive coupling. Direct coupling is most widely used, easy to operate and is based on the insertion of electrodes into the cell culture medium to deliver ES. The use of an electrospun, poly-L-lactide/polyaniline fiber scaffold in the presence of ES showed neurite extension [89]. However, challenges in biocompatibility, pH, and other areas have raised questions about long-term cell survivability. Capacitive coupling is a comparatively biocompatible approach and provides uniform distribution of the electric field among the cells onto the scaffolds. It also does not require conductive scaffolds. Finally, inductive coupling uses a regulated electromagnetic field using a conductive coil which surrounds the cell culture system. This setup is also known as pulsed electromagnetic field stimulation (PEMF). PEMF replicates the human body system in transmitting stimuli [90]. Inductive coupling has shown promising responses in osteogenic differentiation [91].

From the perspective of regenerative medicine platforms such as wound healing and tissue regeneration and repair, directional cell migration and alignment are promising features of cells. The regulation and enhancement of such features is worth exploring in order to understand the mechanisms involved. As a biophysical factor, ES has shown promising results in activating specific cell signaling pathways and in inducing cell migration and alignment. The significant effect of cell alignment and the redirection of cells to be aligned has been seen in cells such as cardiac, adipose tissue-derived progenitor cells, endothelial progenitor cells, vascular ECs, and BMSCs, and along with changes in cell alignment, cell directional growth was observed with changes in the direction of ES [92]. One study has shown the use of parallel versus perpendicular field vectors and changes in cell alignment. PC12 cells showed reorientation in perpendicular field vectors and showed enhanced neurite extension in parallel field vectors compared to perpendicular vectors [93].

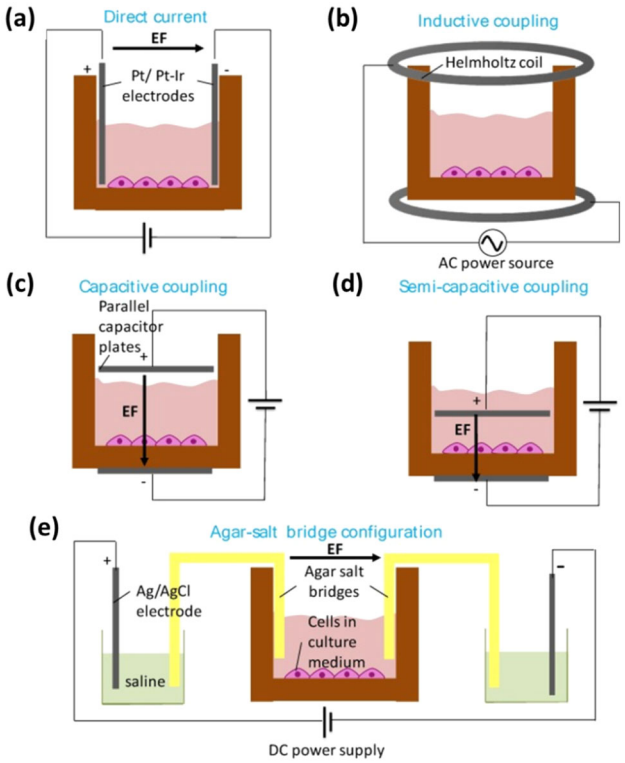


Fig. 7 Different approaches of electrical stimulation (ES) application in in vitro cultures: **a** direct current (DC) stimulation utilizes parallel electrodes coupled directly by the culture medium; **b** inductive coupling (IC) creates ES from oscillating electromagnetic fields generated by passing alternating currents; **c** capacitive coupling (CC) produces ES by using two metallic/conducting plates placed above and below the cell culture dishes; while in **d**, one of the plates (usually the top plate) is immersed into the culture medium in the case of semi-capacitive coupling; **e** in the agar salt-bridge configuration, the electrodes are immersed in electrolytes in separate chambers and connected to the cell culture dish. Reprinted from [94], Copyright 2018, with permission from Elsevier

Conductivity effects on cell proliferation, differentiation, and maturation

Along with the impact of ES on cell migration, it has been shown that ES can impact cell proliferation, differentiation,

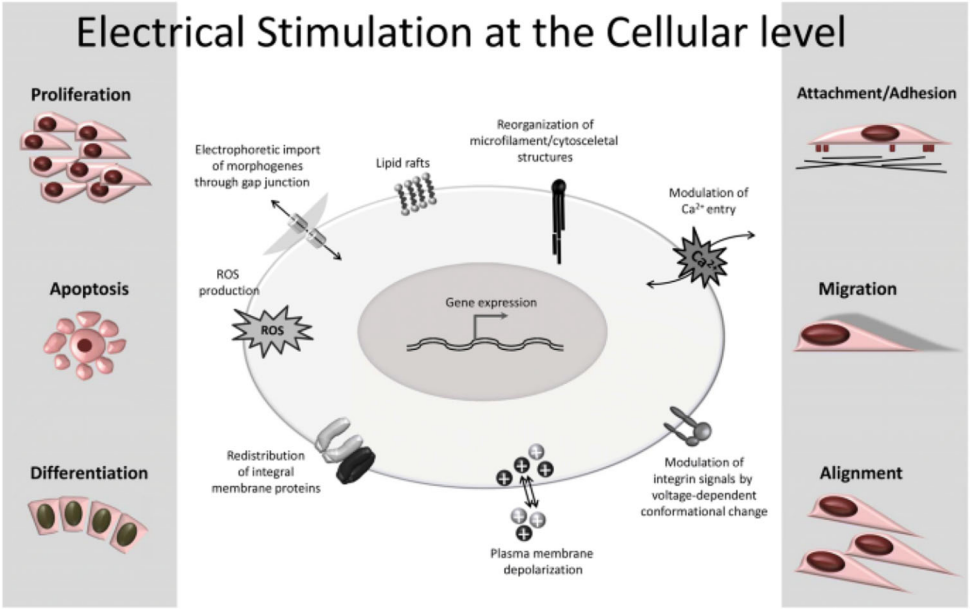


Fig. 8 The impact of electrical stimulation on cellular mechanisms and functions. Reproduced with permission from Ref. [91]

and maturation. Attaining the cell's functional properties to repair and regenerate the lost and/or damaged tissue still persists as a major challenge in regenerative medicine. Technologies and platforms which could enhance cells' proliferative and differentiation abilities show promise in regenerative medicine as depicted in Fig. 8. With an increase in ES within the intensity range, cells have shown enhancement in the rate of proliferation. Cells such as preosteoblasts, osteoblasts, human umbilical vascular endothelial cells, NSCs, and human dermal fibroblasts have shown up to a 1.5 times increment in cellular metabolic activity and proliferation, with negligible phenotypic changes [95]. Shorter stimulation periods with high intensity have also shown promotion in cell proliferation. However, a higher intensity of ES could lead to cell death. Intercellular mechanisms, Ca^{2+} related pathways, cellular alignment, proliferation, and even differentiation could be impacted by ES to enhance tissue engineered scaffolds composed of metallic biomaterials, electroactive polymers, and carbon-based materials [3]. Brief ES drives the human-induced pluripotent stem cells to cardiac differentiation, while a continuous preconditioning leads to enhanced cardiomyocyte maturation and function [96]. ES along with specific growth factors promotes the differentiation and mineralization of the osteoblastic cell line of MC3T3-E1 cells, demonstrated by increased alkaline phosphatase activity and extracellular calcium deposition [97]. Osteoblast-like Saos-2 cells cultured and subjected to ES on a substrate made of biodegradable polylactide and electrically conducting PPy resulted in mineral deposition high in calcium and phosphate content and significantly upregulated osteoblast-specific markers, such as ALP, BMP2, and Runx2 [98]. Neural stem cells cultured on electrospun conductive nanofibers, consisting of 15% polyaniline (PANI) and PCL/gelatin, when stimulated electrically for an hour, significantly showed longer neurite outgrowths as compared to unstimulated cells [99].

Future prospects and conclusions

The electroconductive properties of the biomaterials used in the biofabrication of electrically active tissues such as nerves and skeletal and cardiac muscle could provide a significant cue for cell function. In addition to spatial and geometric biomimicry, the addition of a conductive element within a structured and hierarchical 3D matrix can further improve the ability of the cell to adhere and undergo functional maturation. Besides biomimicry, the physical properties of electroactive materials—such as stiffness, elastic modulus, interfacial adhesion, and porosity—also provide mechanical support for bioprinted tissue constructs [8].

Though a wide range of biomaterials has been used for cell-laden bioprinting, the diverse demands on conductivity and on chemical, biological, and biomechanical characteristics as described in the previous section have been satisfied by very limited biomaterials. In particular, very minimal biomaterials and bioink options are available that can exhibit optimal mechanical properties, biocompatibility, conductive properties, and effective ES, and provide the complex cellular structural environment as found in the native tissues. More research is therefore required in basic and translational biomedical studies to establish novel conductive biomaterials for the formulation of bioinks.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

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